

PRX- B6N #1 ES Cells

These cells were derived and expanded on primary mouse embryonic fibroblasts (**MEFs**) in medium containing **1000U/ml LIF** and cultured at **7.5% CO₂** in humidified air at 37°C.

- Replenish medium daily
- Passage every other day
- Trypsinize gently using 0.05% trypsin/EDTA
 - (HyClone # SH30236)

DAY 1: Thaw one vial of ESC directly into a 25cm² flask containing a confluent layer of inactivated MEFs and 5.5mls of freshly prepared ESC medium.

Note: Thaw a vial quickly in a 37°C water bath by gently shaking it until all the frozen material has thawed. Spray the vial with 70% ETOH and wipe with kimwipe. Aseptically transfer the contents of the vial into the prepared 25cm flask, which has been equilibrated in the incubator for 1-2 hours prior to thawing the ESCs.

DAY 2: Examine the cells under the phase contrast scope at 4x and 10x. ESC colonies should be visible. Depending on the density of the colonies, either replace the medium and return the culture to the incubator OR passage the cells to a 10cm dish containing a confluent layer of inactivated MEFs.

DAY 3: Examine the cells under the scope. If the cells were not passaged to a 10cm dish, do so today. If they are already on the 10cm dish, depending on the size and density of the colonies, either replace the medium OR trypsinize the culture and freeze $\frac{2}{3}$ to $\frac{1}{2}$ in 2 cryogenic vials for future experiments and passage the remaining $\frac{1}{3}$ to $\frac{1}{2}$ of the cells to a fresh 10cm dish for electroporation in 20-24 hours.

PRX-B6 ESC MEDIUM

Final volume: 200ml

Component	Amount	Final concentration	Vendor	Catalogue number
IMDM (Iscove's DMEM)	154ml	~80%	HyClone	SH30259.02
FCS- ESC qualified**	40ml	20%	HyClone	SH30070.03 E
NEAA (MEM) 100x	2ml	1x	HyClone	SH30038.01
L glutamine (200mM)	2ml	2mM	HyClone	SH30034.01
Pen/strep	2ml	1x	HyClone	SV30010
B-Mercaptoethanol	3ul	0.2mM	Sigma	M-7522
ESGRO- mouse LIF	20ul	1000U/ml	Chemicon	ESG1107

Mix all ingredients in the top of a 2micron PES filter unit and filter. Store at 4°C. Discard any unused medium after 10 days.